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TERMINAL REPORT

covering the period

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MECHANISM OF ENZYME ACTION

Prepared by

C. G. Overberger, Thomas St. Pierre, Norman Vorchheimer
and Samuel Yaroslavsky

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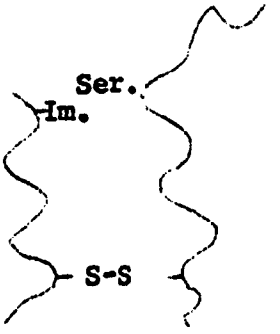
INTRODUCTION

The estrolytic catalysis of chymotrupsin is polyfunctional, involving, at a minimum, the imidazole group of histidine and the hydroxyl group of serine. Since even small aliphatic esters are affected, close proximity of the two functional groups is required. As demonstrated by degradation studies, this condition is not realized through a close existence of the two amino-acids on the same chain, but rather by a proper coiling of the chymotrypsin molecule, or even by a mutual interaction of two different molecules, held together by -S-S- bonds:[1]

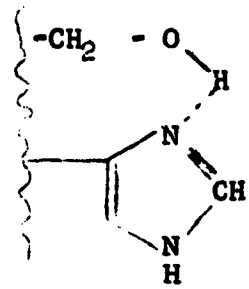


The exact nature of the interaction between the two functional groups when brought together has not been elucidated, and even the sequence of steps involved in the catalysis is still debated. For the acylation steps two main schemes are being offered[2,3]:

a) "Nucleophilic catalysis": a preliminary attack of imidazole on the ester's carbonyl with subsequent transfer of the acyl residue to the serine hydroxyl.

b) "General basic catalysis": a direct acylation of the serine hydroxyl, which is being activated by the neighboring

imidazole group. This activation is probably a hydrogen bonding type rather than a neutralization reaction. Hydrogen bonding requires a juxta position of the imidazole and alcohol groups.



The geometric patterns play a role not only in the interrelations of the different functional groups on the catalyst but also in the interaction between catalyst and substrate. This is very well demonstrated by the group of phenomena that fall under the category of "neighboring group effects." It is at least partially responsible for the selectivity displayed by enzymes.

One of the purposes of the study of polymeric models for enzymatic activity is to discern these geometric contributions from electrostatic and other factors which may have an equally important role. Comparative determination of rate profiles coupled with dissociation data and related measurements provide an analytical tool for the understanding of the intricacies of the mechanism of polymeric catalysts. The information obtained may be utilized for the improvement of the model systems in comparison with the natural catalysts.

Results

Poly 4-vinyl imidazole and poly 5-vinyl benzimidazole have been prepared and their catalytic activity with p-nitrophenyl acetate has been compared to that of monomeric imidazole and benzimidazole.

Rates were studied in a Beckman (model DU . spectrophotometer thermostated at 26°C, by measuring the absorption of p-nitrophenol liberated as a function of time.

Solutions were buffered with tris-(hydroxymethyl) aminomethane (TRIS) and hydrochloric acid. The catalytic rate was calculated according to the following equation

$$k_{cat} = \frac{k_{obs} - k_{blank}}{C_{(catalyst)}}$$

The results for poly 4-vinyl imidazole, the synthesis of which is described in earlier reports[4], are summarized in Tables I and II. In Table II the kinetic runs were kept at constant ionic strength (0.02).

TABLE I (4)

Catalytic Rate Constants of Imidazole and Poly 4-Vinyl Imidazole

pH	k_{cat} (ℓ/mole min.) Poly 4-vinyl imidazole	Imidazole
7.0	4.4	5.4
8.2	24.4	13.2
9.0	75.5	18.6

Substrate - p Nitrophenyl Acetate
Subst./Catalyst - 1/1
Solvent - 28.5% Ethanol/Water
Buffer - 0.05M TRIS

TABLE II

Catalytic Rate Constants of Imidazole and Poly 4-Vinyl Imidazole

pH	k_{cat} (ℓ/mole min.) Poly 4-vinyl imidazole	Imidazole
7.2	9.1	11.4
8.2	21.4	15.0
9.0	44.2*	17.8

*Substrate/catalyst - 1/1

Substrate - p Nitrophenyl Acetate
Subst./catalyst - 1/10
Solvent - 28.5% ethanol/water
Buffer - 0.02M TRIS
Ionic Strength - 0.02

The results of poly 5-vinyl benzimidazole which was prepared directly from 5(β-chloroethyl)benzimidazole[5] are summarized in Table III

TABLE III

Catalytic Rate Constants of Benzimidazole and Poly 5-Vinyl Benzimidazole

pH	k_{cat} (ℓ/mole min.) Poly 5-vinyl benzimidazole	Benzimidazole
9.1	2.1	0.4
9.6	15.0	2.4
10.4	36.4	27.7

Substrate - p Nitrophenyl Acetate
Subst./Catalyst - 1/10
Solvent - 30% n-Propanol/Water
Buffer - 0.02M TRIS
Ionic Strength - 0.02

The fact that at high pH values the polymers are better catalysts than the monomers is unexpected. To test whether these phenomena depend on alterations of pK values, ultraviolet titration[6] of both poly 4-vinyl imidazole and poly 5-vinyl benzimidazole were performed (see figs. 1 and 2).

In 28.5% EtOH solution and 0.1M KCl the apparent pK_1 of poly 4-vinyl imidazole was found to be 6.6 (the pK_1 of imidazole in 28.5% EtOH is 6.95)[7]. The empirical relationship between the degree of dissociation α $\left(= \frac{C_{Im}}{C_{ImH^+} + CI_m}\right)$ and pH was found to be

$$pK = 6.6 = pH + 1.6 \log \frac{1-\alpha}{\alpha} \quad (8)$$

By the use of this formula or by direct reading from Figure 1 determination of α at any pH is made possible.

In Figure 3, $k_{cat.}$ of poly 4-vinyl imidazole and imidazole* were plotted against α. The plot shows that the enhanced activity of poly 4-vinyl imidazole cannot be explained by changes in pK_1 only. Decreased pK_2 value for the polymer at certain pH may occur through external interactions.

The ultraviolet titration of poly 5-vinyl benzimidazole was carried out in three different pairs of solvents, i.e. 30% diglyme-water, 30% n-propanol-water and 30% dimethoxyethane-water and 0.01M KCl (figure 2). The apparent pK_1 in 30% n-propanol-water

* α values for imidazole were calculated using the estimated formula $pH = 6.95 + \log \frac{\alpha}{1-\alpha}$

was found to be 3.5 (the pK_1 of benzimidazole in 28.5% EtOH is 5.4)[7]. According to Figure 2, α is practically 1.0 at the pH's studied. In Figure 4, K_{cat} for poly 5-vinyl benzimidazole and benzimidazole in 30% n-propanol-water is plotted against pH. The enhancement of the catalytic rate of the polymer vs. the monomer cannot be explained on the basis of pK_1 only.

While the protonated sites of the polymers are inactive towards neutral substrates they may serve as centers of attraction for charged substrates[9]. 3-Nitro-4-acetoxybenzoic and served as substrate for these experiments. The results are summarized in Table IV, and plotted versus α in Figure 5 (the dotted part is only estimated as yet, and has to be substantiated by experiment).

At pH 7.4 it has been found that salicylic acid inhibits the rate of hydrolysis of 3-nitro-4-acetoxy benzoic acid. The inhibition is very probably due to the occupation of the binding sites by the salicylic acid. A Michaelis-Menten treatment of these phenomena will be attempted in order to find out to what extent the absorption phase contributes to the enhanced activity of the polymer. A similar treatment is also planned for discerning the absorption forces in the case of the neutral substrate.

TABLE IV

Catalytic Rate Constants of Imidazole and Poly-4-Vinyl Imidazole

pH	$k_{cat.}$ (1/mole min.)	
	Poly 4-vinyl imidazole	Imidazole
2.4	0	0
7.2	120.2	24.4
8.2	104.4	31.9
9.0	55.5*	34.5

* Subst./Catalyst - 1/1

Substrate - 3-Nitro-4-acetoxybenzoic Acid

Subst./Catalyst - 1/10

Solvent - 28.5% Ethanol/water

Buffer - 0.02M TRIS

Ionic Strength - 0.02

Figure 1

pH vs α for Poly 4-Vinylimidazole

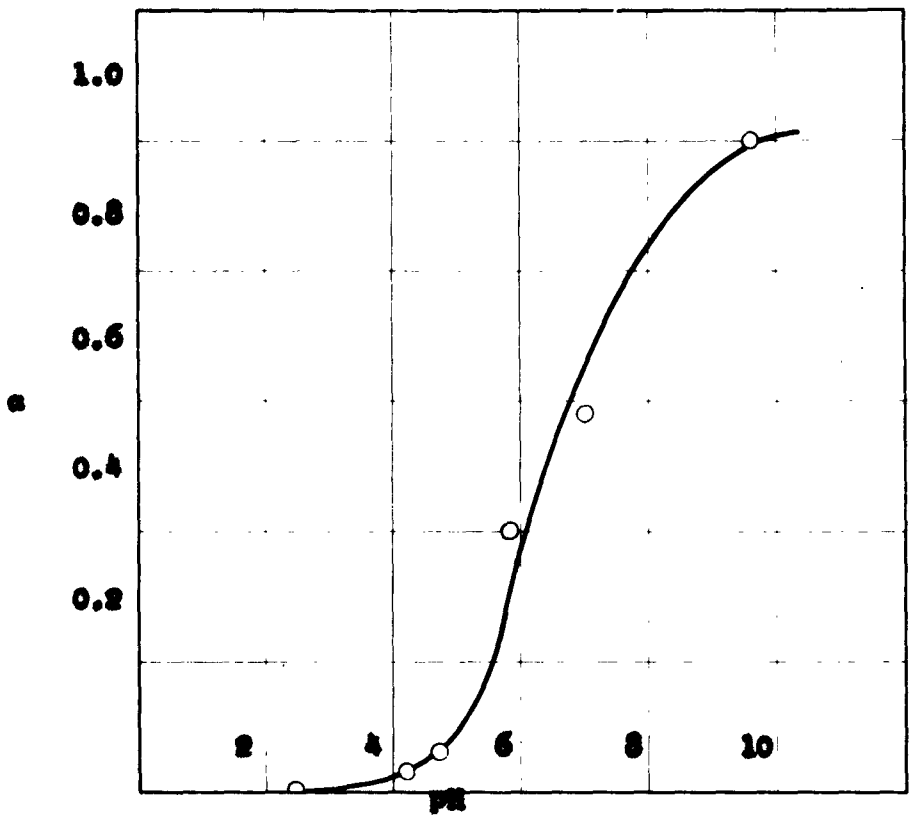
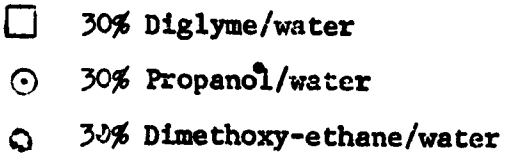

1.0
0.8
0.6
α
0.4
0.2
2
4
6
8
10
pH

Figure 2

pH vs α Poly 5-Vinylbenzimidazole



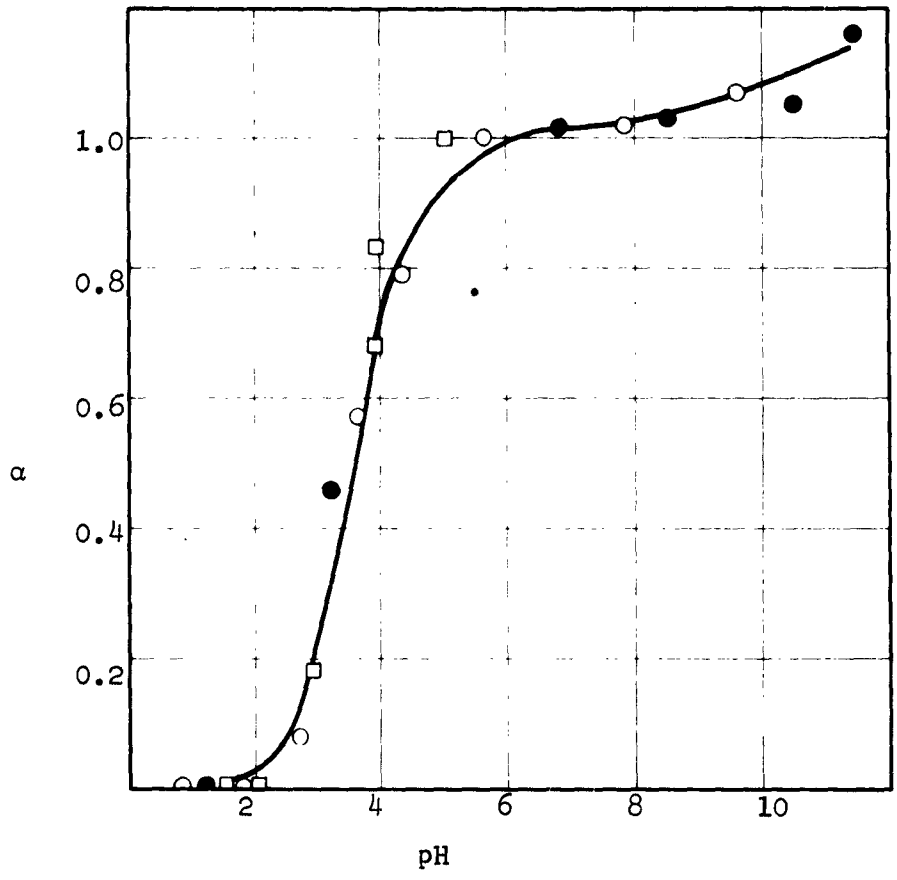

1.0
0.8
0.6
0.4
0.2
α
2
4
6
8
10
pH

Figure 3

k_{cat} vs α for p-Nitrophenyl Acetate

● Poly 4-Vinylimidazole

○ Imidazole

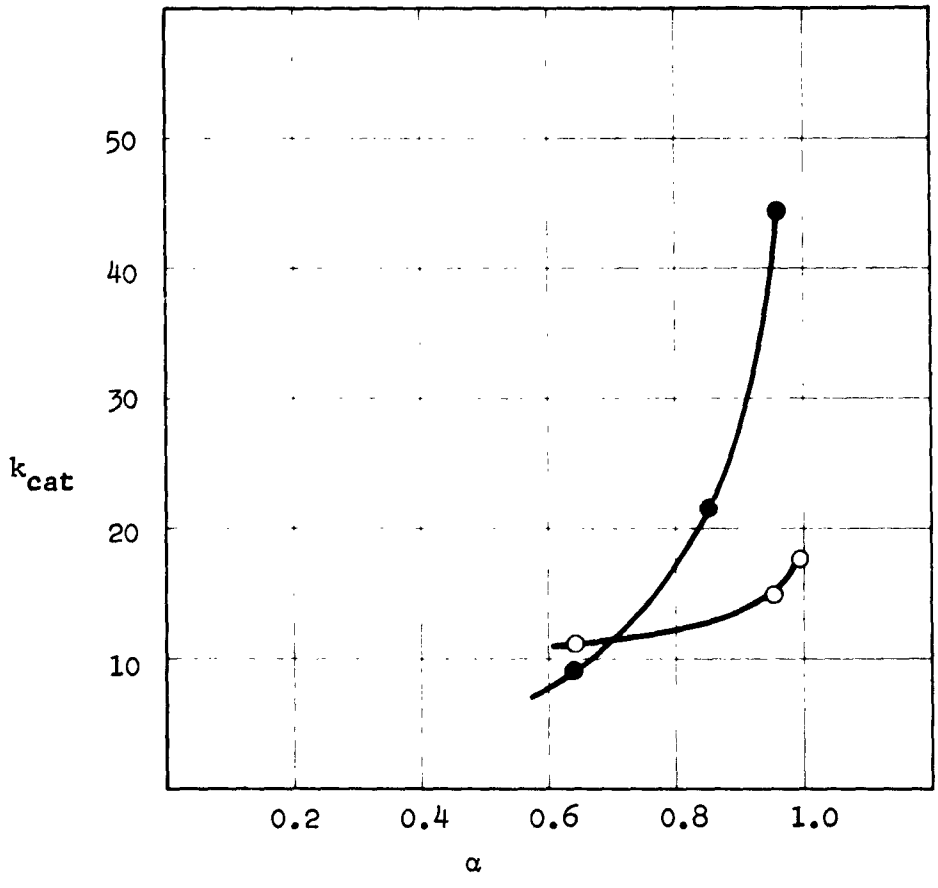

50
40
30
20
10
k_{cat}
0.2
0.4
0.6
0.8
1.0
α

Figure 4

K_{cat} vs pH for p-Nitrophenyl Acetate


Poly 5-vinyl benzimidazole


Benzimidazole

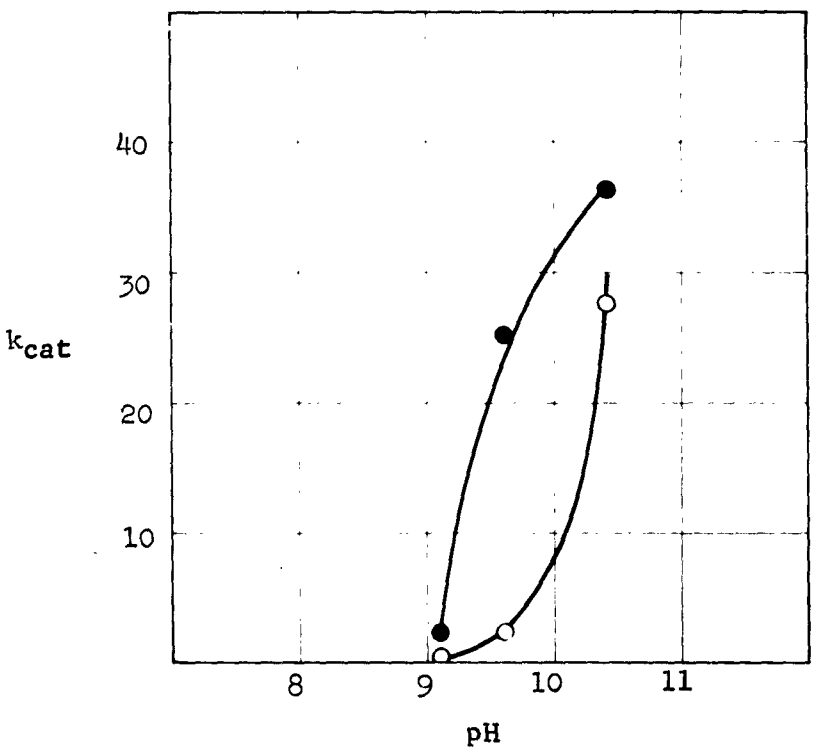
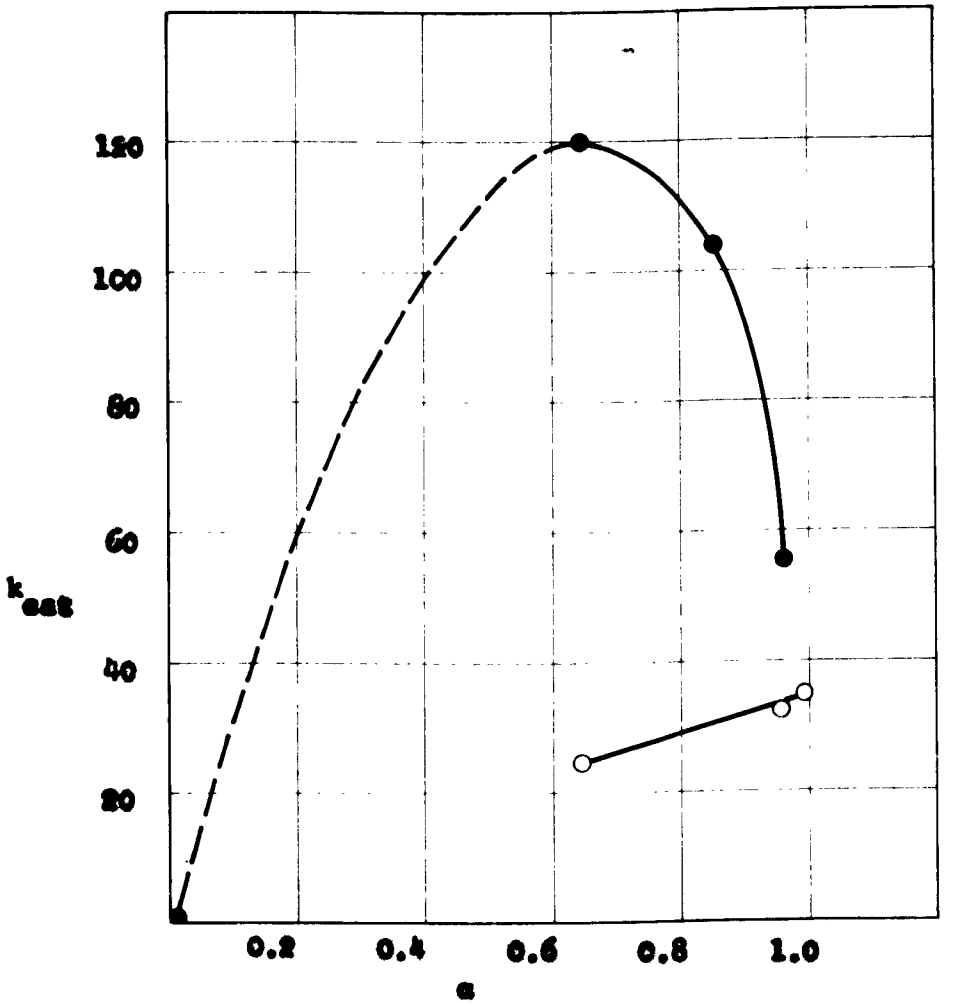

40
30
20
10
k_{cat}
8
9
10
11
pH

Figure 5

k_{cat} vs α for 3-Nitro-4-acetoxybenzoic Acid

● Poly 4-vinylimidazole

⊙ Imidazole


120
100
80
60
40
20
k_{cat}
0.2
0.4
0.6
0.8
1.0
α

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